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An introductory review on humic substances: aspects of research on their genesis, their physical and chemical properties, and their effect on organisms

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Abstract

Some properties of humic substances and the turnover of soil organic matter are discussed as an introduction to the study of humus and nitrogen reserves in soils. Brief and concise comments are given on the chemistry of the humic substances. A chemical explanation of their colour is also proposed. Examples of humus content and soil fertility are given. The influence of some constituents of soil organic matter on plant growth is differentiated into two effects: direct and indirect. The uptake of phenolcarboxylic acids, as examplified by vanillic acid labelled at different positions with ^{14}C, is described as well as the ensuing catalytic influence on some metabolic pathways of plants. These compounds are sought as exerting a favourable effect on plant growth. The effect seem to be enhanced when other growth promoting factors – e.g. temperature, humidity, light intensity, oxygen saturation of liquid culture medium, moisture content of soils as well as nutrient supply and budget – are sub-optimal. From an economical point of view the results presented indicate a partial depression by these products of the variability of crop yields.

Formation of humic substances by transformation of plant residues

Because humus is a dynamic system, it continually changes as its constituents decompose and are formed anew. The diverse processes involved are influenced by the conditions of soil formation, namely the plant cover, the activity of microorganisms and animals, the climate, the chemical and physical properties of the soil, and human activity. The combination of these factors determines the state of soil organic matter as indicated by the amount and composition of the humus, its distribution in the profile, the properties of the humus substances, and their interactions with the mineral components of the soil. Some authors have attempted to show relationships between the conditions for soil formation and the properties of soil organic matter. The work on the characterization of the total humus components of soil and particularly humic and fulvic acid fractions has been reviewed by Kononova (1966). The results of this work are schematized in Fig. 1. Some of the properties investigated and utilized to construct the figure are most strongly developed in deep chernozem (soil No. 5); these properties (upper diagram) include the following:

1. humus reserves,
2. reserves of nitrogen (mainly organic),

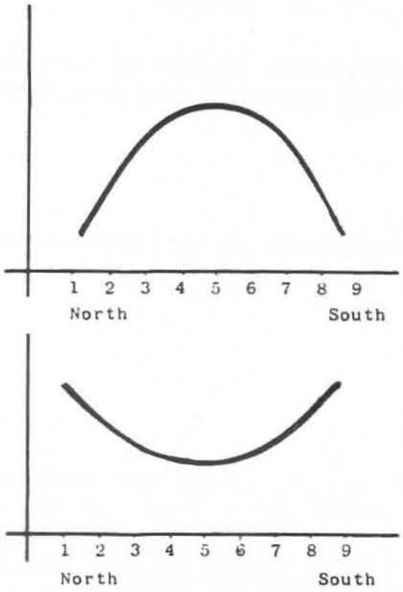


Fig. 1. Measurements of properties of soil organic matter of different soil types of the USSR (according to Kononova, 1966).
1 = podzolic soil; 2 = light grey; 3 = dark grey, podzolic forest soil; 4 = degraded; 5 = deep; 6 = ordinary chernozem; 7 = dark; 8 = light chestnut soils; 9 = serozem.

3. humic acid (HA content),
4. ratio of HA to Fulvic Acid (FA),
5. carbon content of HA,
6. the C:H ratio, a measure of the 'aromaticity', of the humic fraction,
7. absorbance due to solutions containing 1 g/l,
8. exchange capacity,
9. number of micro-organisms per g of soil.

Other properties which show minimal development in the same soil type are shown in the lower portion of the same figure:

1. FA content,
2. hydrogen content of HA.

All these investigations were more or less related to problems of soil genesis. Furthermore, it must be borne in mind that these systematic investigations concern the soils in the USSR, ranging from North to South, and can not be generalized. More subtle and detailed distinctions are necessary for the description of humic systems in individual soils, for comparison of humic substances and for explanation of the functions of humic systems in bioproduction (Salfeld, 1965, 1971; Salfeld & Söchtig, 1971).

Turnover of organic matter in soil

Usually organic matter does not accumulate in the soil under natural conditions. After a period of time, annual additions of organic material in the soil come to be in balance with the annual losses of soil organic matter. Thereafter the organic matter content of a soil remains constant. This process, in which the losses equal

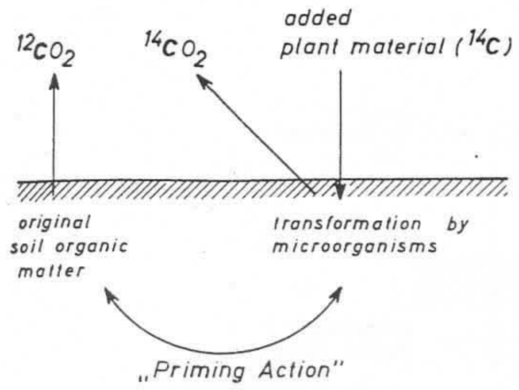

$^{12}CO_2$
$^{14}CO_2$
added plant material (^{14}C)
original soil organic matter
transformation by microorganisms
„Priming Action"

Fig. 2. Balance between increase and degradation of soil organic matter.

the gains, is described as turnover and may be defined as 'the flux of organic material through the organic matter in a given sample of soil' (Jenkinson, 1966).

The rate of addition and the rate of decomposition are likely to be changed when the environmental conditions of a soil are changed by bringing a virgin soil under cultivation or by altering a system of agriculture. Ultimately a new balance between losses and gains will be reached, the soil organic matter attaining a new equilibrium level.

The formation and decomposition of organic matter – especially humic substances – have been investigated by addition of plant material uniformly labelled with carbon-14. By comparative study of labelled and unlabelled carbon dioxide given off, not only could the transformation of the added plant material by microbial action be followed for a longer period of time, but also the simultaneous decomposition of the organic matter which had been present in the soil previously could be monitored (Fig. 2). When readily decomposed organic matter is present in the soil prior to the addition of the labelled plant material, its accelerated decomposition material is observed. This phenomenon is called 'priming action'. The turnover of organic matter is influenced, moreover, by climatic conditions, the system of agriculture, the plant associations, the soil pH, the chemical composition of soil constituents, and the mechanical properties of the soil. Thus, many factors affect the turnover of organic matter in soil and therefore the formation of humic substances.

Nitrogen transformation in soil organic matter

The nitrogen cycle (Fig. 3) can be investigated in different ways. A detailed treatment of the role of humus formation and decay in the terrestrial nitrogen cycle will be given by Jansson in this meeting. For our purpose it should be mentioned that only a small active fraction participates in the mineralization – immobilization cycle, a large fraction being inert and therefore not included in this cycle.

The amount of available nitrogen is a limiting factor for the degradation of dead plant material during its transformation to humic substances as well as for soil productivity.

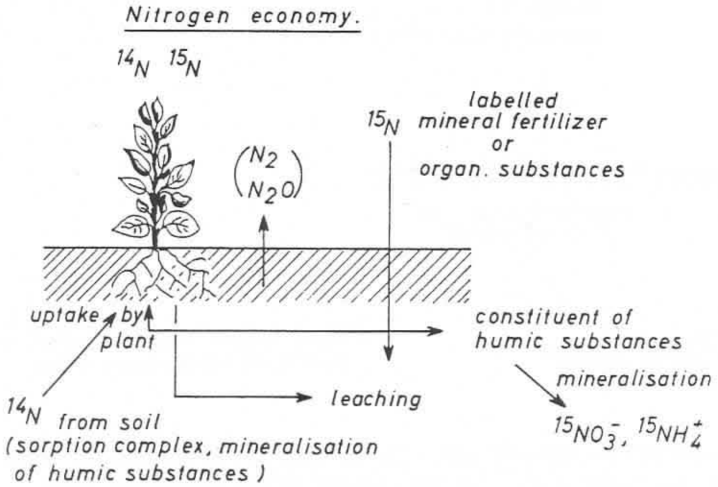

Nitrogen economy.
^{14}N ^{15}N
^{15}N labelled mineral fertilizer or organ. substances
N_2 N_2O
uptake by plant
constituent of humic substances
mineralisation
leaching
^{14}N from soil (sorption complex, mineralisation of humic substances)
$^{15}NO_3^-$, $^{15}NH_4^+$

Fig. 3. Nitrogen transformation in soil organic matter.

The addition of easily available nitrogen salts accelerates the biochemical degradation of plant material. The decomposition of holocellulose is more greatly accelerated than the decomposition of lignin (Fig. 4). Therefore, the possibility exists that lignin degradation products participate directly in the formation of humic substances. If one isolates the lignin fractions from decaying plant material with sulphuric acid, the concentration of methoxyl groups decreases and the nitrogen content increases as a function of the time of rotting (Fig. 5; Broadbent, 1954; Bartlett & Normann, 1938; Bartlett, 1939; Flaig, Schobinger & Deuel, 1959; Ritter, Seborg & Mitchell, 1932; Flaig, 1960a; Waksman & Smith, 1934; Stöckli,

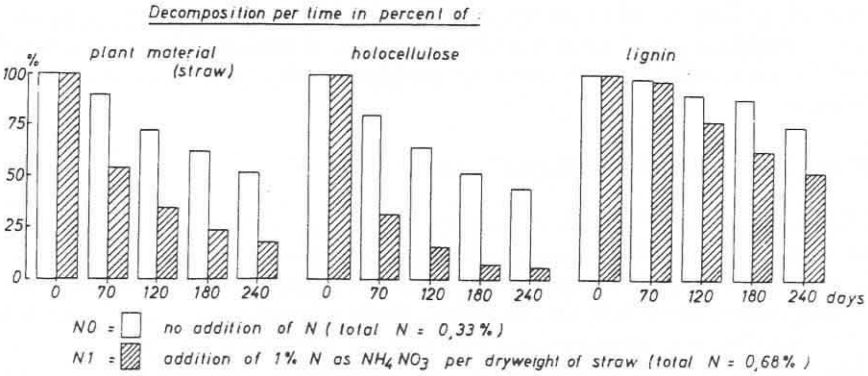


Fig. 4. Decomposition per unit of time in percent of plant material (straw), holocellulose, and lignin.

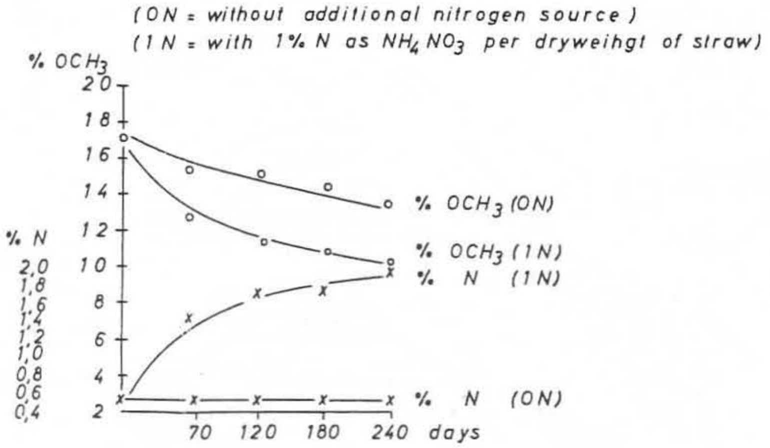


Fig. 5. Alterations of nitrogen and methoxyl content of the lignin fractions of straw during humification.

1952; Nehring, 1955). This suggests that the cleavage of the methoxyl group precedes the introduction of nitrogenous groups (Flaig, 1960b).

The fixation of nitrogen compounds after alteration of several functional groups in the lignin preserves their molecular structure and renders the nitrogen less accessible to micro-organisms.

Summarizing comments on the chemistry of formation of humic substances

The scheme shown in Fig. 6 is concerned only with selected problems of formation of humic substances and is not intended to embrace all processes of humification. The scheme shows that there are two essential sources of phenols for the formation of humic substances in nature.

1. The formation of phenols from lignin occurs mainly by microbial degradation, whereby the cleavage of C-C bonds and of ether linkages plays an important role. The structure of the lignin molecule is thereby disrupted. Degradation products of various sizes are formed, and these contribute to the formation of the darkcoloured humic substances by reaction with nitrogenous compounds derived from protein and its degradation products in all phases of degradation. Methylether cleavage and increase of oxygen content play an important role in nitrogen fixation.
2. The microbially synthesized phenols, which are chiefly of the resorcinol and phloroglucinol type, contribute to the formation of humic acids after transformation by hydroxylation to phenols, which in turn are oxidizable to quinones, and after reactions with nitrogenous compounds, or by oxidative coupling with quinone.
3. It is not known to what extent the nitrogen-free polymers of lignin degradation products participate in the formation of humic substances. They are available carbon sources for micro-organisms.

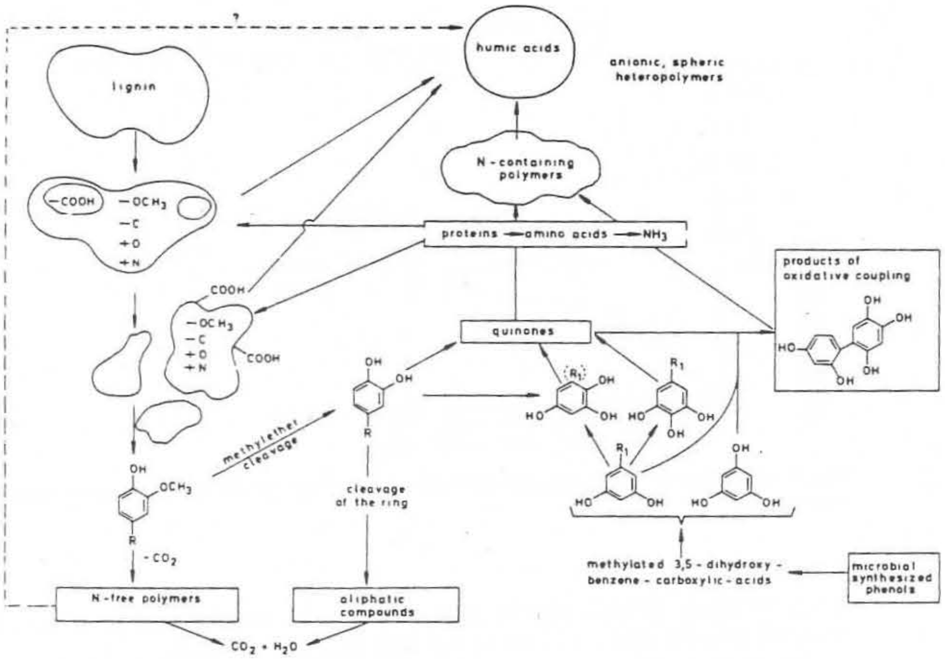


Fig. 6. Contribution of phenolic and nitrogenous structural units to the chemical composition of humic substances.

4. Some of the phenolic compounds derived from lignin or synthesized by micro-organisms disappear from the mixture of compounds, they participate in the synthesis of humic substances by cleavage of the benzene ring and formation of aliphatic acids. These can be used as carbon sources of micro-organisms.
5. The scheme demonstrates the various possible reactions and types of substances which occur in the humic system as a whole.

Many questions remain to be answered. It is not yet clear for instance, which of the condensation products of phenolic and nitrogenous compounds are most stable in soil. The products in question are lignin molecules which have suffered alteration of reactive groups and which have condensed with nitrogenous compounds, or polymers containing nitrogen and formed by polymerization of lignin degradation products and other phenolic compounds. The availability of the organically bound nitrogen is important for soil productivity.

The colour of humic acids

Intramolecular quinhydrones as models of humic acids

We performed some model experiments to explain the absence of discrete maxima in the absorption curves of humic acids (Fig. 7). According to its u.v.

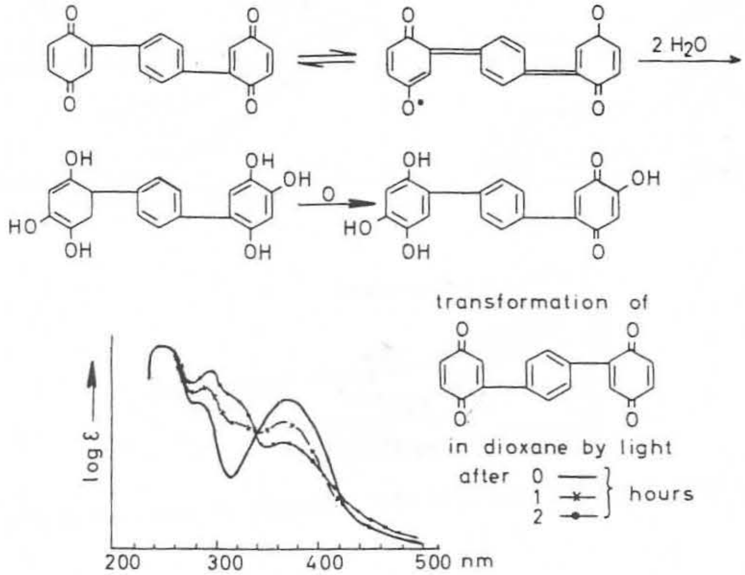


Fig. 7. The transformation of 1,4-diquinonyl benzene: an example of the use of polymeric quinones as models of humic acids.

spectrum, 1,4-diquinonyl-benzene seems to have an *o*- and *p*-quinonoid configuration in solution (Ploetz, 1955). The *o*-configuration may be defined as biradical and is very reactive. A corresponding hydroxyhydroquinone is formed by addition of water. This can be partially reoxidised, and a deeply coloured intramolecular quinhydrone is formed. Diquinonyl-benzene is disproportionated in organic solvents. The mixtures of substances thus formed show similar, nearly monotonic spectra increasing in absorbance with decreasing wavelength, as with humic acids isolated from soils. (Viz. Frömel, 1938a, 1938b, 1941) and later on many others.

Steelink and Tollin (1962) and Steelink (1964) demonstrated by electron paramagnetic resonance that humic acids contain radicals. This results agrees well with the assumption that the humic acids are intramolecular quinhydrones. Measurements by Kleist and Mücke (1966) and by Kleist (1967) established that the radicals (semiquinones) are stabilized by mesomery and are responsible for the colour of humic acids. Thus, the humic acids should have the properties of electron exchangers.

Influence of nitrogen on electronic spectra of humic acids

In Figure 8 the absorption spectra of different humic acids and fulvic acids are depicted schematically. The curves are not linear, as could be shown by more exact measurements (Salfeld, 1965). As a rule, humic acids from chernozems, which are richer in nitrogen, not only absorb light more strongly, but also absorb more strongly at longer wavelengths than the humic acids from podzols, which are poorer

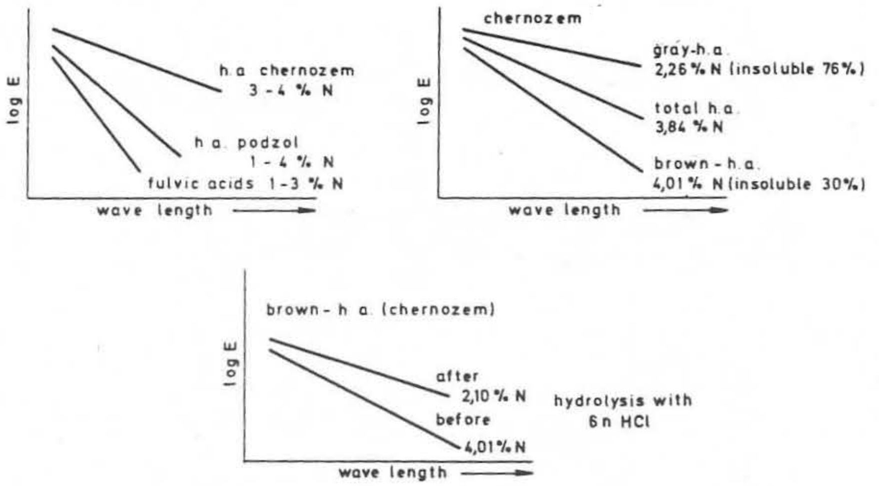


Fig. 8. Absorption spectra of humic acids from chernozem and podzol and of fulvic acids (schematic).

in nitrogen. The stronger absorption of humic acids from chernozems may perhaps be caused by the higher content of non-hydrolysable nitrogen, which may have a higher concentration of bathochromic heterocyclic compounds, compared with podzol humic acids. The total nitrogen content can not play an important role in light absorption, since it is largely in the form of proteins, which do not absorb in the visible range.

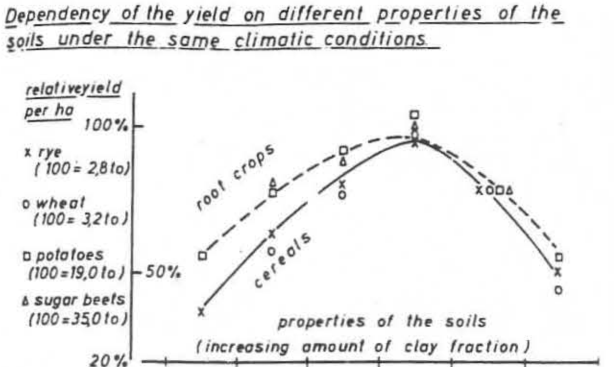
The brown humic acid fraction of chernozems has a higher nitrogen content than the gray humic acid fraction. However, light absorption by the gray humic acid fraction is stronger in the total range of wavelengths than that of the brown humic acid fraction, although the nitrogen content of this fraction (4.01% N) is higher than that of the hydrolysis residue (2.01% N).

This theoretical consideration of the colour of humic substances has been developed according to results of chemical investigations, but it must be supplemented by further investigations and isolation of the compounds in question.

Humus content and soil productivity

The production capacity of soils for crops depends on their composition (Fig. 9). In the graph the constituents of soil are divided into sand (inorganic particles in the size range 0.05–2 mm in diameter), clay (inorganic particles less than 0.05 mm in diameter), and humus.

The figure shows that the yields of cereals and of root crops depend on how these soil constituents are combined. Humus medium loam (inorganic fraction between 0.05 and 0.01 mm = 58%, fraction smaller than 0.01 mm = 33%) had the most favorable composition for crop production.



relative content:	sand	high loamy sand	heavy sandy loam	humus medium loam	heavy loam	clay soil
humus	o	+	+	++	+	o
sand	+++	++	+	+	o	o
clay	o	+	++	++	++	+++

o = relative small amount; +, ++, +++ = increasing amounts

Fig. 9. Dependence of crop yield on different properties of soil under constant climatic conditions.

To convey some idea of the participation of humus in the production of crops, the yields obtained from various soils different in humus content are compared. According to the crop yield, the productivity of soils increases as a function of humus content.

Increased yield may occur under both favourable and unfavourable climatic conditions (Table 1). It is not yet clear to what extent the quantity of humus or particular humus fractions on the one hand, and the quality of certain humus constituents on the other, cause the increased yield. Therefore, not only the characteristics of certain fractions but also the total humic system must be characterized to get information about the possible relationship between humus and crop production. Jenny et al. (1968), in a large scale experiment investigated the relationship between soil organic matter and soil productivity on the one hand and state factors and soil properties on the other as a multivariate system. The correlation coefficients representing the relationship between soil carbon and other soil properties were greatly influenced by their dependence on precipitation (e.g. rainfall). Correlation of yields with organic constituents measured by the carbon content of soils are high. It was found that N or C alone or in combination account for about 63–64% of the yield variance.

Using a soil map of the world, Bramao (1968) dealt with the question of the influence of soil organic matter on soil productivity. He established a correlation between the organic carbon content of soils and crop yield, showed that the yield of wheat is more greatly influenced than that of maize. Both authors discuss the problem of the importance of quantity and quality of soil organic matter and possible differences in the evaluation of both these actors.

Table 1. Yield (t/ha) and yield increase (%) of crops in various types of soil when the soil is enriched in humic matter, both under favourable (F) and unfavourable (U) climatic conditions.

Soil type	Inorganic fraction (%)		Rye				Potatoes			
	0.05–0.01 mm	< 0.01 mm	F		U		F		U	
			yield	increase	yield	increase	yield	increase	yield	increase
Sand	10	9	1.15		0.9		11.5		10.0	
Sand, humic	10	9	1.35		1.08		13.5		11.5	
				7		6		9		7
Loamy sand	12	14	1.50		1.14		14.7		12.3	
Loamy sand, humic	12	14	1.74		1.42		16.5		13.4	
				8		5		8		5
Mild loam	58	33	2.56		2.46		21.0		17.2	
Mild loam, humic	58	33	2.78		2.62		22.0		18.0	
				8		7		4		9

Direct and indirect effects of soil organic matter on plant growth

Depending on environmental conditions, humic substances can have a more or less pronounced effect on growth and yield of plants. The uptake of inorganic ions can be influenced by humus in different ways. One may draw a distinction between direct and indirect effects (see Fig. 10; and also Flaig & Söchtig, 1962).

The indirect effect is characterised by the fact that the organic substances are not taken up by the plant. The processes in this category occur outside of the plant and are concerned mainly with alterations of chemical and physical properties of the soil.

A direct effect occurs when the organic substances are taken up by the plant through the roots and participate in the plant's metabolism.

The organic substances in the soil can be divided into high-molecular weight and low-molecular weight substances (Fig. 11).

The incentive to classify organic substances in soil according to molecular weight results from the fact that for the most part only substances of low molecular weight (approximately 1 000) are taken up by plants, while substances of high-molecular weight are almost never taken up. The carbohydrates, uronides, and nucleic acids, which are mainly linear colloids, and the proteins, chitin, and humic acids, which are spherical colloids, are considered the most important high-molecular weight substances. Low-molecular weight compounds are formed by hydrolysis or oxidative degradation of the high molecular substances. In addition, low-molecular weight compounds formed by the metabolism of the living organisms are found in the soil. As a rule, the fulvic and hymatomelanic acid fractions of humic matter consist mainly of low molecular weight compounds.

On the whole it can be said that the high-molecular weight compounds effect alteration of the physical properties of soils (texture, porosity, etc.), whereas the low-molecular weight substances participate in chemical reactions in the soil and changes in the metabolism of plants after absorption by the roots.

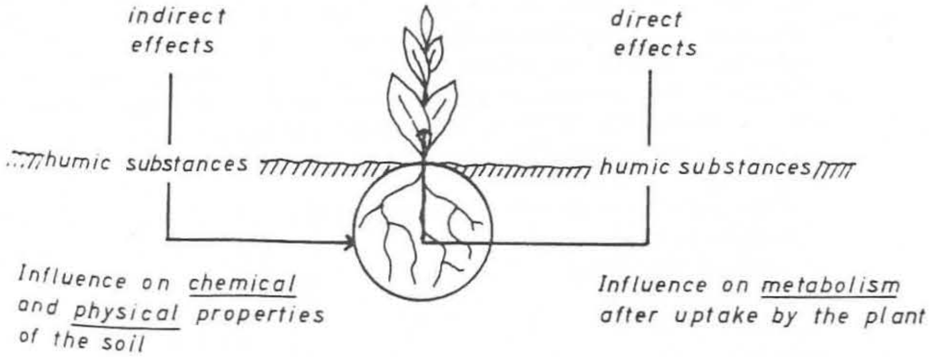

indirect effects
direct effects
humic substances
humic substances
Influence on chemical and physical properties of the soil
Influence on metabolism after uptake by the plant

Fig. 10. Scheme of possible effects of humic substances on growth and productivity of plants.

	soil organic matter	
high molecular weight (mol. wt $\geqslant 10^3$)		*low molecular weight*
(1) linear colloids	carbohydrates uronides nucleic acids	(1) hydrolysis products and degradation products by oxidation
(2) spheric colloids	proteins lignins chitin humic acid	(2) metabolites of organisms (3) fractions of fulvic, hymatomelanic acids
	indirect effects	
alteration of physical properties of soils		participation in chemical reactions in soil
	direct effects	
none		influence of metabolism of organisms

Fig. 11. Classification of organic substances in soil according to their effects on plants.

Retention of pollutants as an indirect effect

In this presentation no more will be written about the indirect effects, such as the influence of organic matter on soil structure, water management, exchange capacity, chemical weathering, redox processes, etc., which are covered. The higher the carbon content of a soil, the stronger is the adsorption of all types of pesticides. Not merely 'Disulfoton', (diethyl-*S*-2-ethyl-thio)-ethylphosphorthiolthionate as exemplified in Figure 12. Cation exchange capacity and clay content do not correlate as significantly with adsorption as does organic carbon content.

Different authors found that decomposing plant material in the field (Williams, 1968) or in model experiments (Walker & Crawford, 1968) adsorb higher amounts of pesticides per gram of soil carbon than does more highly humified organic material in soil. Some authors (Hance, 1969; Süss & Wagner, 1969) assume that the humic acids as such are not the constituents of soil organic matter that are most effective in the sorption of pesticides. Furthermore, the degree of sorption is a function of chemical structure. The sorption and possible consequent stabilization or slow release of these compounds are still poorly known and would be important subjects of future research.

Sullivan and Felbeck (1968) tentatively propose a mechanism for the adsorption of atrazine by alcoholic extracts of humic acids. (Fig. 13). Observed changes in the infra-red spectra following formation of the atrazine-humic acid complex specifically, changes in the bands for phenolic OH (3 μm), aliphatic C-H (3.4 μm), C=0 (5.8 μm), -C-N- (6.15 μm) and COO (7.2 μm) of *s*-triazine, suggest that the carboxylic and phenolic hydroxyl groups of the humic acids interact with the nitrogen atoms of the alkyl-amino substituted *s*-triazines.

Properties of soils

No.	pH	CEC meq/100g	Clay %	Organic carbon %
1	81	6 3	8	0 7
2	81	10 4	18	0 9
3	68	10 2	6	1.1
4	68	10 6	6	1.3
5	75	13 0	26	1 4
6	80	21 9	36	1 7
7	81	10 6	10	1 7
8	72	14 0	21	1 8
9	6 5	10 7	7	1 8
10	78	19 8	1 8	2 7
11	63	18.2	2	2.8
12	75	44 8	11	7.6
13	77	48.2	16	8.8
14	76	74.0	36	11 0
15	72	66.4	25	12.0
16	51	83.4	62	15 0
17	6.9	118 8	peat soil	31 0

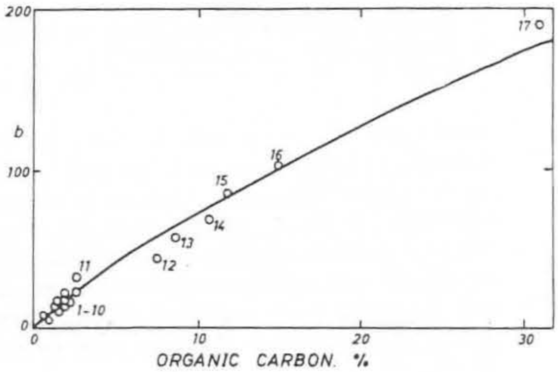


Fig. 12. Relationship between organic carbon content of soil and adsorption of pesticides (Graham-Bruce, 1967).

Uptake of phenolic substances as a direct effect

Some of the important direct effects are listed in Table 2. Of all these effects only the fourth, the catalytic effect of organic components from soils on the

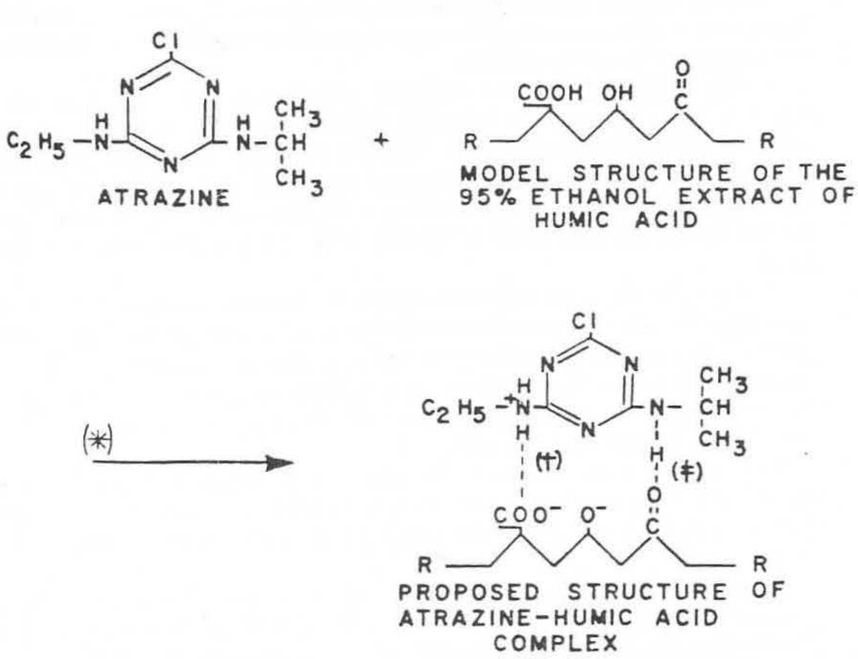

ATRAZINE
MODEL STRUCTURE OF THE 95% ETHANOL EXTRACT OF HUMIC ACID
(*)
(†)
(‡)
PROPOSED STRUCTURE OF ATRAZINE-HUMIC ACID COMPLEX

Fig. 13. The mechanism tentatively proposed for the adsorption of atrazine by soil humic acids (Sullivan & Felbeck Jr, 1968).

Table 2. Direct effects on soil organic matter on plant growth

1. Better adsorption and distribution of heavy metals as complexes.
2. Influence on ion adsorption.
3. Direct use of absorbed organic substances for formation of plant protoplasm.
4. Catalytic effect on metabolic processes.

metabolism of plants, will be discussed here, because its contribution to the formation of plant biomass is less well known that in the case of the others. From many papers (Blanchet, Chaminade, Khristeva, Guminski, Prat & coworkers, Niklewski, Rypacek, Sladky, Saalbach, Söchtig, Tichy, quoted by Flaig, 1965, 1968) it is known that fractions of humic substances can have a favourable effect on growth and yield in nutrient solutions, sand cultures and in some cases also in soil. However, some published results do not confirm the favourable effect of humus or its fractions. We have tried to elucidate these contradictions (Söchtig, 1964).

The following pages describe experiments demonstrating the absorption by plant roots of phenolic substances formed during decomposition of plant residues. The substances absorbed may be fractions of soil organic matter or well defined compounds such as phenol carboxylic acids.

Phenolcarboxylic acids as physiologically active substances in soil organic matter

Of the various lignin degradation products, only those compounds will be mentioned which seem to be important for our discussion of the uptake of organic compounds from soil by plants.

During lignin degradation different compounds are formed by biochemical reactions (Fig. 14). Most of the lignin degradation products have a carboxyl group and are phenol derivatives with different numbers of methoxyl groups in the *o*-position with respect to the hydroxyl group and with side chains having one or three carbon atoms in the *p*-position with respect to the hydroxyl group. The shortening of the side chain of phenolacrylic acids occurs mainly at the double bond.

Demythylation is an important reaction, whereby gallic acid is formed from syringic acid, and protocatechuic acid from vanillic acid. From protocatechuic acid aliphatic keto-carboxylic acids are formed by cleavage of the ring. Hydroxylation is another reaction which occurs during transformation of lignin degradation products. *p*-Hydroxybenzoic acid is hydroxylated to protocatechuic acid, which in turn is hydroxylated to gallic acid.

Hydroquinones are formed in the course of oxidative decarboxylation catalysed by phenoloxidases. Vanillic or syringic acid is transformed to methoxyhydroquinone and 2,6-dimethoxyhydroquinone after application to plants (Harms et al., 1971).

All the above mentioned compounds are transformed to dimers or polymers of different types by dehydrogenation. Several other transformations of other phenol carboxylic acids are known. To some extent the physiological activity of phenolic compounds depends on their molecular properties.

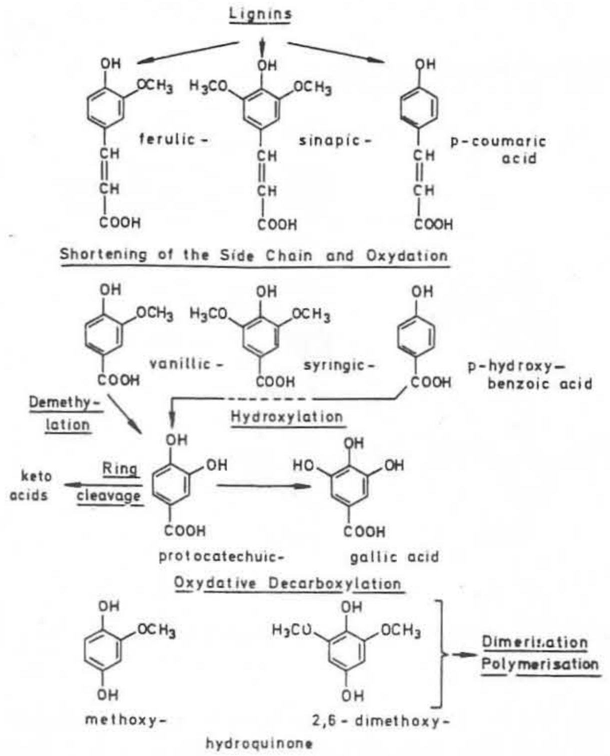


Fig. 14. Transformation of lignin degradation products.

Uptake, transport and transformation of phenol carboxylic acids in plants

To study the uptake, transport, and transformation of phenol carboxylic acids, differently labelled phenolic compounds shown in Figure 15 have been used.

The phenol carboxylic acids were marked with carbon-14 in the carboxyl group (-c-), in the methoxyl group (-m-) or uniformly in the ring (-r-). The compounds were added to sterile cultures of wheat seedlings in nutrient solutions. The incubation times were three or six days, or more.

The transport occurs in the acropetal, basipetal and transversal directions in the case of monocotyledons (wheat seedlings) or dicotyledons (mustard seedlings) Fig. 16).

Some of the compounds used were found to be secreted into the nutrient solution through the roots, or distributed throughout the plant after application to different organs as the diagram in Figure 16 demonstrates. After glucosidation of the phenol carboxylic acid, transport was no longer observed. The rate of uptake, the transport of the compounds in the plant, and the rate of decarboxylation are influenced by the substitution with one or two methoxyl groups.

As an example, the results of application of variously labelled vanillic acids are given in Figure 17.

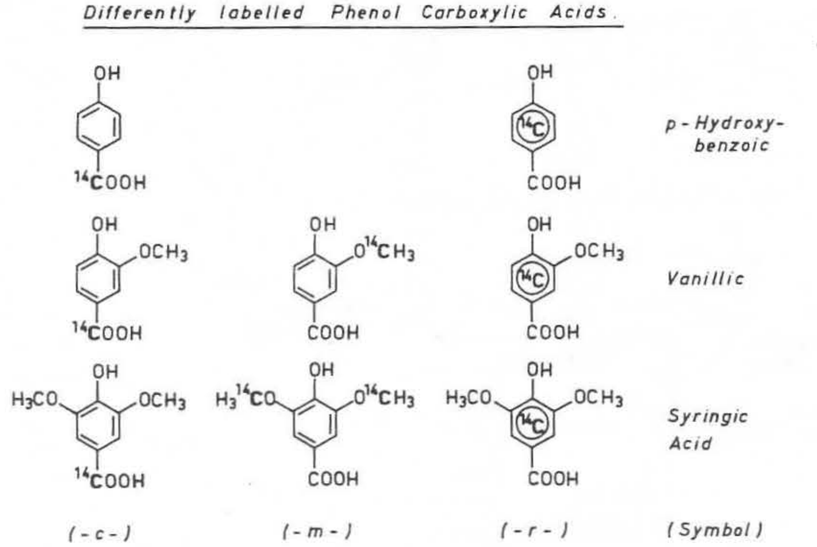


Fig. 15. Phenolic compounds with carbon-14 labelling in different molecule positions, used in physiological experiments with plants.

The total radioactivity of the sprouts and roots in µg is smaller after addition of carboxyl-labelled vanillic acid than after methoxyl- and ring-labelled vanillic acid. But if one includes the activity of the carbon dioxide released, the amount is nearly the same. In order to determine the distribution of labelled carbon in the various

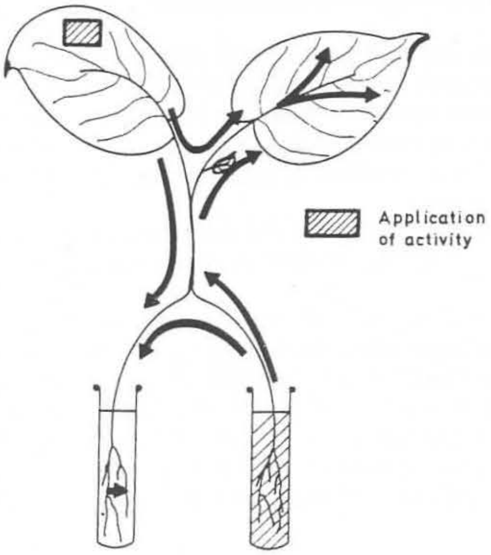


Fig. 16. Distribution of labelled phenolic compounds in plants.

plant constituents, the sprouts and roots were extracted with solvents of different dissolving capabilities. The activity in the different extracts was distributed as follows:

1. In the ether extracts a relatively small part of the activity could be identified as free phenolic acids.
2. The greatest amount of activity was in the methanol extracts of the plant organs. By physical and chemical tests it could be demonstrated, that the major part of activity belonged to the glucose esters, glucosides or their glucose esters of the vanillic acids (Harms, Söchtig & Haider, 1969b).
3. The activity of the water extract of the sprouts belongs mainly to metabolites such as amino acids, soluble proteins, and carbohydrates which were formed by endogenous fixation of radioactive carbon dioxide generated by decarboxylation of the carboxyl-labelled phenol carboxylic acids.
4. The activity in the residues of water extraction of the sprouts in the case of carboxyl-labelled vanillic acid was also caused by the fixation of carbon dioxide: but the labelled products were high-molecular weight plant-constituents such as cellulose, proteins, and lignin. After hydrolysis with 6 *N* hydrochloric acid most of the activity was in the amino acids aspartic and glutamic acid, which are formed by amination of oxalo-acetic acid and 4-ketoglutaric acid formed in the citric acid cycle.
5. The activity in the residues of extraction of the residues of the roots is mainly due to formation of condensation products formed from the added phenolic compounds.
6. The lower activity in the residues of the roots in the case of addition of carboxyl-labelled vanillic acid is evidently due to the participation of decarboxylated and therefore inactive substances in the formation of as yet unidentified

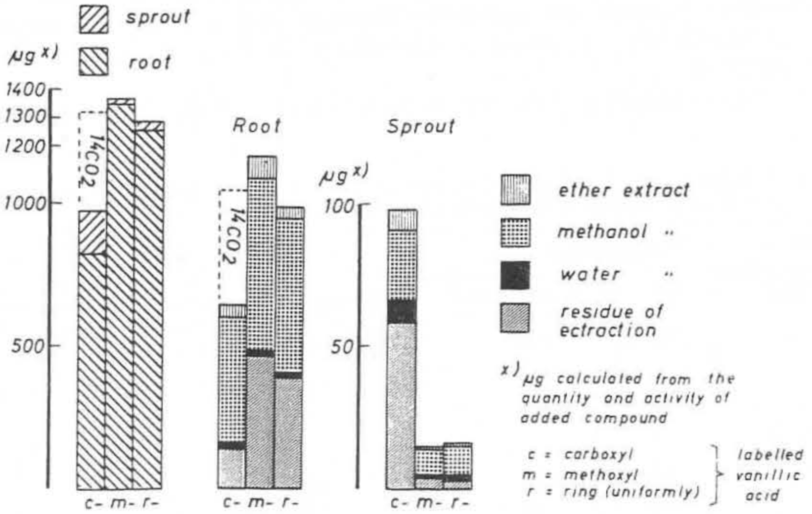


Fig. 17. Amount of radioactivity in different extracts of sprouts and roots after 6 days' incubation with variously labelled vanillic acid (Harms et al. 1971).

polymeric substances. This conclusion is supported by the fact that the total activity reaches the same levels as in the other cases when one adds the activity of the released carbon dioxide to that which is determined in the roots.

7. Methoxyhydroquinone, the decarboxylation product of vanillic acid, was identified among the products, and it accounted for 1% of the radioactivity. This result is important in that the physiological activity of the hydroquinones is about 100 times higher than that of the phenol carboxylic acids. Therefore, it is possible that the observed alterations of plant metabolism are caused more by the hydroquinone than by the phenolic acid.

Influence of phenol carboxylic acids on plant productivity

In this section only the effect of the above-mentioned biochemically-active substances on increase or decrease of the productivity of plants in different stages of their life cycles will be discussed.

In sand cultures and in experiments with Mitcherlich pots the addition of phenol carboxylic acids increased the dry weight of the rye seedlings or the yield of grains of spring wheat under certain environmental conditions. (Table 3). The influence on plant growth could be observed mainly at the beginning of the growth period. In a similar way we investigated various other substances, such as phenols, quinones, growth substances, and fractions of humic substances (Flaig & Saalbach, 1958).

Dependence of the effect of humus constituents on plant growth on environmental conditions

Many publications have established that fractions of humic substances can have a favourable effect on the growth and yield of plants in nutrient solutions, sand cultures, and in some cases also in soil. But some published results do not confirm the favourable effect of humus or its fractions. We have tried to elucidate these contradictions.

It must be emphasized that the degree of influence of these metabolically-active

Table 3. Influence of phenol carboxylic acids on the relative dry weight of rye seedlings (sand culture) and the relative yield of summer wheat (Mitcherlich pots).

Acid concentration (mol/l)	Rye seedlings				Summer wheat			
	p-hydroxycinnamic acid		vanillic acid		protocatechuic acid		vanillin	
	sprout	root	sprout	root	grain	straw	grain	straw
0	100	100	100	100	100	100	100	100
10	116[1]	103	123[1]	130[1]	·	·	·	·
6×10^{-5}	·	·	·	·	111[1]	104	113[1]	108[1]
12×10^{-5}	·	·	·	·	110[1]	113[1]	111[1]	111[1]
10^{-5}	118[1]	103	117[1]	126[1]	·	·	·	·
10^{-6}	95	100	117	118	·	·	·	·

1. Statistically significant.

substances depends on environmental conditions such as temperature, light and humidity, as well as on nutrient supply. Thus, Chaminade (1965) found that toxic overdoses of inorganic nitrogenous salts remain effective as far as plant production is concerned in the presence of humus, whilst in the absence of humus a decrease in production occurred. The experiments were performed in pots using rye grass grown in sand cultures, and the nitrogenous source was ammonium sulfate (Fig. 18).

Guminski and Guminska (1953) investigated the influence of humus fractions on the production of biomass of tomatoes at low oxygen tension in water cultures. The yield of unaerated nutrient solution was higher in the presence than in the absence of humic substances. Nevertheless the yield was the same in aerated cultures with and without the addition of humic substances (Fig. 19).

In pot experiments Söchtig (1964) (Fig. 19) investigated the influence of thymohydroquinone, a model substance analogous to an oxidised lignin degradation product. The soil was kept water-saturated, and the water flow was about 100 ml. The leaching of nutrients was not influenced by the addition of thymohydroquinone. In spite of unfavourable effects such as leaching of nutrients, water saturation of soil, and low oxygen tension, the hydroquinone increased the yield remarkably (up to 13 g), but not up to the level of the control (29.2 g), which did not suffer leaching of nutrients and water saturation of the soil. Further observation of favourable effects have been made in the case of deficient light and water; in the latter case the wilting resistance is increased.

The experiments mentioned demonstrate that the effect of a metabolically-active substance on plant growth depends on the environmental conditions. The favourable effect of humus on plant growth and yield is explained schematically in Figure 20 (Söchtig, 1964).

When growth factors such as temperature, humidity, light, oxygen tension of the culture medium, and water saturation of the soil, as well as the supply or balance of nutrients, are 'optimal', the yield is the highest.

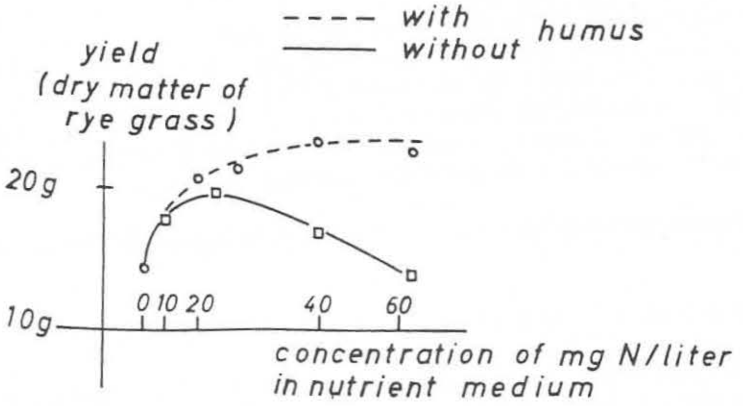


Fig. 18. Influence of humic substances on productivity of ryegrass following administration of an overdose of inorganic nitrogenous salts (after Chaminade, 1965).

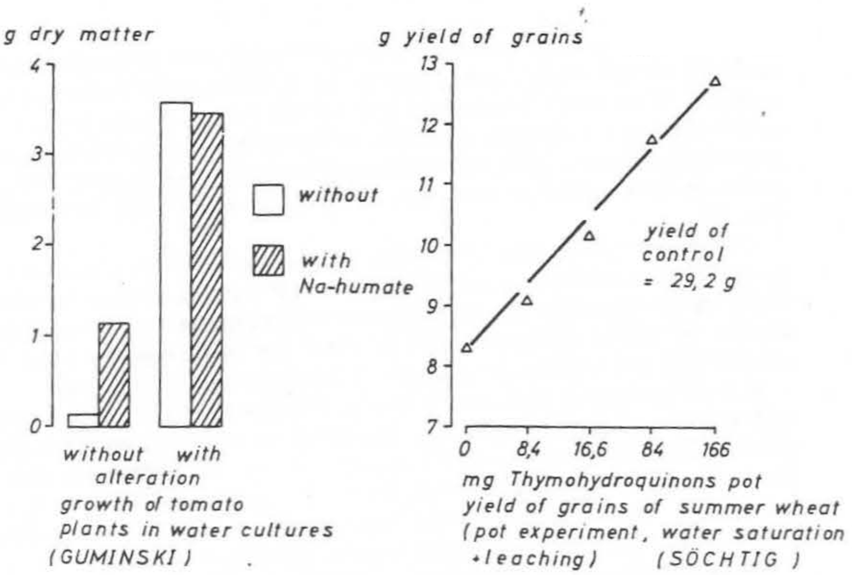


Fig. 19. Results of experiments on the effects of oxygen deficiency in the presence of humic matter in nutrient cultures (Guminski) and on the leaching by water in the presence of thymohydroquinone (Söchtig).

Enhancement of productivity by humic substances depends on whether one or more growth factors are deficient or in excess. If the effects of humic substances and lignin degradation products are seen from this perspective, the contradictory results in the literature can be explained.

Economically, the phenomena mentioned mean a partial diminution of the risk of yield variance or, in other words, a stabilization of production. However, some of the environmental conditions, such as climatic factors (heavy rainfall, dry seasons, etc.) cannot be predicted.

These experimental results can also explain, in part, some observations of the farmers on the 'humus effect'. The physiologically active substances contribute only to a part of the most favourable effect of humus constituents. If these effects were better understood, we might be able to apply our knowledge to practical problems. Only small amounts of such substances would be needed, because they are effective in quantities on the order of kilograms per hectare.

Possible importance of bioregulating effects of humus constituents for crop production

Next, I shall try to explain why humus may contribute to the economy of crop production.

From the past to the present enormous increases of crop production per hectare were caused by use of mineral fertilizers, by plant breeding, by the use of pesticides and herbicides, and also by soil cultivation. In the future, along with factors such as

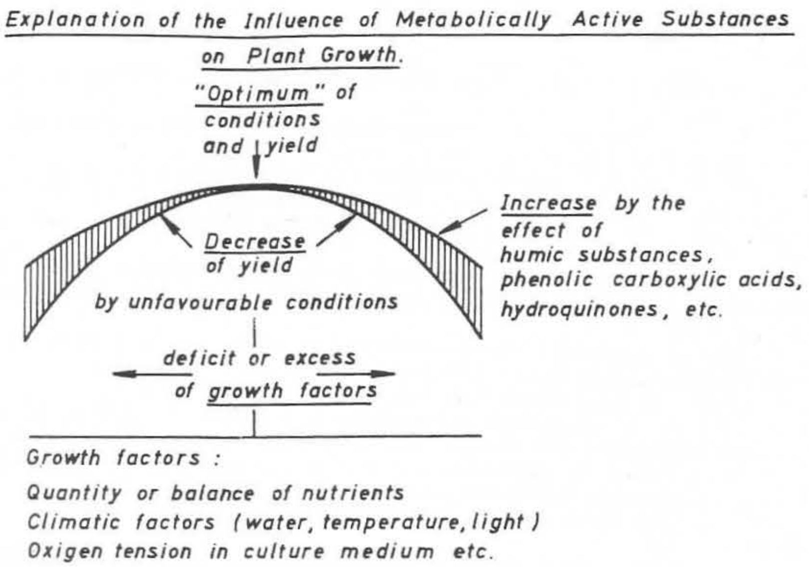

Explanation of the Influence of Metabolically Active Substances on Plant Growth.
"Optimum" of conditions and yield
Decrease of yield
by unfavourable conditions
deficit or excess
of growth factors
Increase by the effect of humic substances, phenolic carboxylic acids, hydroquinones, etc.
Growth factors :
Quantity or balance of nutrients
Climatic factors (water, temperature, light)
Oxigen tension in culture medium etc.

Fig. 20. Influence of physiologically active substances on plant productivity under different environmental conditions.

new production systems like hydroponics, or new plant varieties, which had not previously been used for nutrition of human beings, or, e.g., cattle, bioregulators will play an important role in further increases of yield per hectare (Fig. 21). Some bioregulators as trichlorocholinchloride (CCC) are used to decrease the stem length in wheat or to slow the release of organic nitrogen fertilizers. As demonstrated above, certain humus constituents also act as bioregulators. But there may exist some other possibilities which are not yet known.

From an economic point of view it would be important to study the effect of bioregulators under unfavourable conditions, and to try to prevent the depression

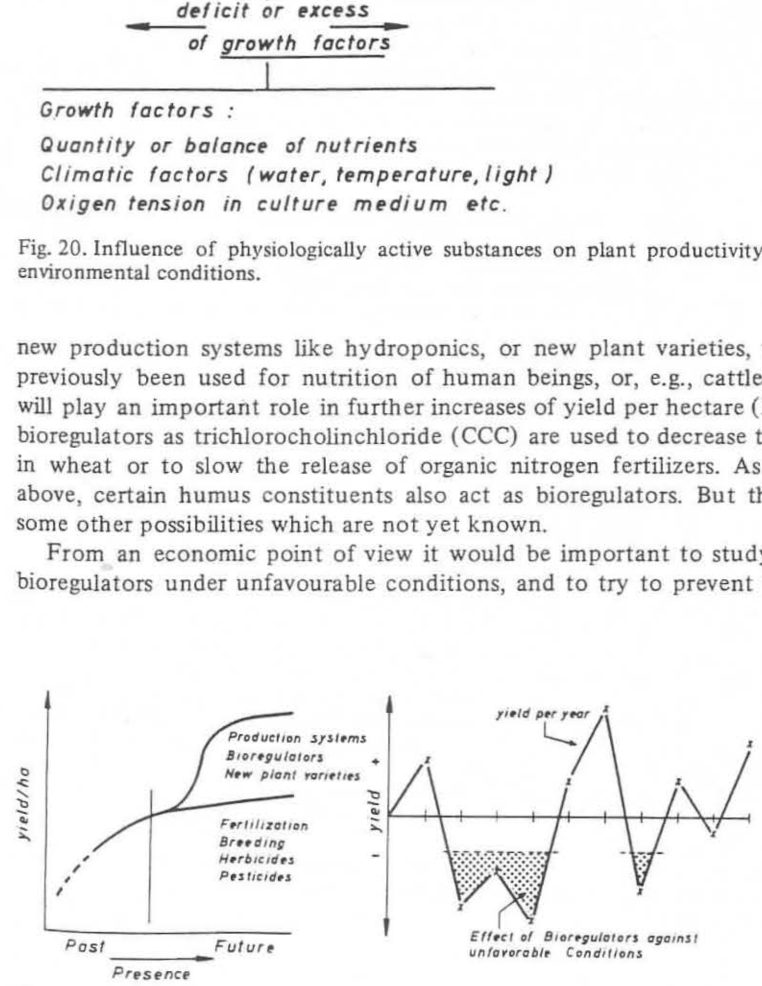


Fig. 21. The level of crop yield in present and future crop production; the importance of bioregulators.

of productivity. Increasing yield by known methods would be the first step. After that, it is to be hoped that basic research in the field of natural and synthetic bioregulators will result in further progress (Fig. 21).

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Discussion

Questioner: A. D. McLaren

Q. Textbooks are out of date: nucleic acids and proteins are easily taken up by plant roots and cause direct effects, such as genetic transformations, leakage of ATP and stunting of root growth. Do you feel that the question of direct uptake and influence of high molecular weight humic substances on plants should be reinvestigated?

A. I did not mention the uptake of high molecular weight substances, because experiments of PRAT with ^{14}C-labelled humic acids, and others by Söchtig, have shown that it is difficult to decide whether humic acids are taken up as such, or if the uptake involves small degradation products of humic acids, products eventually formed during the progress of the experiment. As a matter of fact there are not published experimental data allowing to unquestionably demonstrate that humic acids are transported into the plant. Alterations of plant metabolism, e.g. of phosphorylation, have been observed, but, as far as we were able to establish, they are caused by phenolic compounds of rather small size. However, in humus there may be other compounds which could induce these effects.